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(54) Title: ENCAPSULATED SUPERPARAMAGNETIC PARTICLES

(57) Abstract

Stable, encapsulated superparamagnetic magnetite particles having a narrow particle size distribution with average particle diameters in the range of from 50 Å to 350 Å are prepared by forming an aqueous dispersion of magnetite particles having the above particle size characteristics in the presence of a surfactant, coacervating a mixture of gelatin and a carboxyl containing hydrophilic polymer such as gum arabic to form a thin coating of coacervate on the magnetite particles and crosslinking the coacervate coating with a gelatin hardener such as glutaraldehyde.

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AMENDED CLAIMS
[received by the International Bureau on 4 December 1989 (04.12.89); original claims 1-10 replaced by amended claims 1-8 (2 pages)]

1. A method for preparing stable, coated superparamagnetic magnetite particles having a narrow particle size distribution characterized by forming an aqueous solution of ferric and ferrous salts by adding a mixture of ferric and ferrous salts to water in amounts that provide a molar ratio of ferric to ferrous ion in the range of from 1.6 to 2.4 and in concentrations in the range of from 0.1 to 1 molar; adding to the solution concentrated hydroxide in an amount in excess of 8 moles of OH^- per mole of ferrous ion present in the solution to form a dispersion of fine particles of magnetite; washing the resultant magnetite particles with water until the pH of the magnetite dispersion is within the range of 10–11; depositing a coating of gelatin and a polymeric acid on the particles by removing excess water from the magnetite dispersion that had been washed to said pH of 10–11, to form a concentrated dispersion of magnetite particles, then coating the thus produced particles by adding the resulting magnetite dispersion to an aqueous solution, having a temperature of at least about 40°C, of gelatin having an isoelectric point greater than about 8 and a polymeric acid comprising at least one recurring acid group; and adjusting to coacervation conditions comprising a pH in the range of between about 4.0 and 5.5 and a concentration of the gelatin/polymeric acid mixture of less than about 2% (wt/vol); and then crosslinking.

2. The method of claim 1 which further comprises adding sufficient acid to the aqueous mixture of ferric and ferrous salts to adjust the pH to less than 1.5.

3. The method of claim 1 which further comprises adding from 0.1 to 5% (wt/vol) of a surfactant to the salt solution.

4. The method of claim 1 which further comprises purging the solution of oxygen by bubbling therethrough an inert gas for a period of at least 10 minutes.

5. The method of claim 1 wherein the coacervate is crosslinked with a suitable crosslinking agent for gelatin.

6. Encapsulated superparamagnetic particles having a narrow particle size distribution, the mean diameter of the particles being within the range of from 70Å to 450Å, said particles comprising particles of magnetite having a narrow particle size distribution, the mean diameter of the magnetite particles being between 50Å and 350Å, said magnetite particles having a magnetization of greater than 30 emu/gm and a coercive force of less than 30 Oe, said magnetite particles being encapsulated with a coating of a crosslinked coacervate of gelatin and a polymeric acid.

7. The superparamagnetic particles of claim 6 wherein said polymeric acid comprises recurring acid groups selected from the group consisting of carboxylic acid groups and sulfonic acid groups.

8. The encapsulated particles of claim 6 wherein the magnetite particles have a magnetization greater than 50 emu/gm and a decorative force less than 25 Oe.

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(54) Title: ENCAPSULATED SUPERPARAMAGNETIC PARTICLES

(57) Abstract

Stable, encapsulated superparamagnetic magnetite particles having a narrow particle size distribution with average particle diameters in the range of from 50 Å to 350 Å are prepared by forming an aqueous dispersion of magnetite particles having the above particle size characteristics in the presence of a surfactant, coacervating a mixture of gelatin and a carboxyl containing hydrophilic polymer such as gum arabic to form a thin coating of coacervate on the magnetite particles and crosslinking the coacervate coating with a gelatin hardener such as glutaraldehyde.

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ENCAPSULATED SUPERPARAMAGNETIC PARTICLES

This invention relates to magnetically responsive particles and to their use in systems in which the separation of certain molecules, macromolecules and cells from the surrounding medium is necessary or desirable. More particularly, the invention relates to methods for the preparation of magnetically responsive particles comprising a magnetite core surrounded by a very thin, stable, modified gelatin coating. If desired, a wide variety of organic and/or biological molecules may be coupled to the coating. The particles (coupled or uncoupled) can be dispersed in aqueous media without rapid gravitational settling and conveniently reclaimed from the media with a magnetic field. The process provided herein yields particles that are superparamagnetic; that is, they do not become permanently magnetized after application of a magnetic field. This property permits the particles to be redispersed without magnetic aggregate formation. Hence the particles may be reused or recycled. Stability of the coating of the invention as well as of the covalent attachment of molecules thereto also facilitates the use and reuse of the encapsulated superparamagnetic particles of the invention.

The magnetically responsive particles of this invention may be coupled to biological or organic molecules with affinity for or the ability to adsorb or which interact with certain other biological or organic molecules or with cells. Particles so coupled may be used in a variety of in vitro or in vivo systems involving separation steps or the directed movement of coupled molecules to particular

sites, including, but not limited to, immunological assays, other biological assays, biochemical or enzymatic reactions, affinity chromatographic purifications, nucleic acid hybridization, cell sorting, cell separation and diagnostic and therapeutic uses, including site specific drug delivery and magnetic resonance imaging.

Very small particles (50–350 Å region) of normally ferromagnetic materials are unable to support magnetic domains and are called superparamagnetic. This means that they are weakly magnetic in the absence of an external magnetic field, but upon the application of an external magnetic field, become magnetic and agglomerate readily. The ease with which such particles become magnetized upon application of a magnetic field is directly proportional to their degree of magnetization, measured in emu/gm (electromagnetic units per gram). Their property of becoming demagnetized upon removal of the magnetic field is inversely proportional to their coercive force, measured in Oersteds (Oe). As a practical matter, materials (particles) that have a degree of magnetization of at least 30 emu/gm and a coercive force of less than 30 Oe can be considered superparamagnetic. Generally, the greater the magnetization and the lower the coercive force, the more usefully or "strongly" superparamagnetic the particles become. That is, less magnetic force is required to magnetize them and they lose their magnetic properties more rapidly upon removal of the outside magnetic force. Such particles have found many uses, ranging from mechanical seals and couplings to biological separations.

A detailed review of pertinent prior art may be found in U.S. Patent No. 4,672,040, issued June 6, 1987. As noted therein, the use of magnetic separations in biological systems as an alternative to gravitational or centrifugal separations has been reviewed by B. L. Hirschbein and others, Chemtech, March 1982: 172-179 (1982); M. Pourfarzaneh, The Ligand Quarterly, 5(1): 41-47 (1982); and P.J. Halling and P. Dunnhill, Enzyme Microb. Technol., 2: 2-10 (1980). Several advantages of using magnetically separable particles as supports for biological molecules such as enzymes, antibodies and other bioaffinity adsorbents are generally recognized. For instance, when magnetic particles are used as solid phase supports in immobilized enzyme systems [see, for example, P. J. Robinson and others, Biotech. Bioeng., XV: 603-606 (1973)], the enzyme may be selectively recovered from media, including media containing suspended solids, allowing recycling in enzyme reactors. When used as solid supports in immunoassays or other competitive binding assays, magnetic particles permit homogeneous reaction conditions (which promote optimal binding kinetics and minimally alter analyte-adsorbent equilibrium) and facilitate separation of bound from unbound analyte, compared to centrifugation. Centrifugal separations are time-consuming, require expensive and energy-consuming equipment and pose radiological, biological and physiological hazards. Magnetic separations, on the other hand, are relatively rapid and easy, requiring simple equipment. Finally, the use of non-porous

adsorbent-coupled magnetic particles in affinity chromatography systems allows better mass transfer and results in less fouling than in conventional affinity chromatography systems.

Although the general concept of magnetizing molecules by coupling them to magnetic particles has been discussed and the potential advantages of using such particles for biological purposes recognized, the practical development of magnetic separations has been hindered by several critical properties of magnetic particles developed thus far.

Large magnetic particles [mean diameter in solution greater than 10 microns(μ)] can respond to weak magnetic fields and magnetic field gradients; however, they tend to settle rapidly, limiting their usefulness for reactions requiring homogeneous conditions. Large particles also have a more limited surface area per weight than smaller particles, so that less material can be coupled to them. Examples of large particles are those of Robinson and others, Biotech. Bioeng., XV: 603-606 (1973), which are 50-125μ in diameter, those of Mosbach and Anderson [Nature, 270: 259-261 (1977)] which are 60-140μ in diameter and those of Guesdon and others, [J. Allergy Clin. Immunol 61(1): 23-27 (1978)] which are 50-160μ in diameter.

Ferromagnetic materials in general become permanently magnetized in response to magnetic fields. Materials termed "superparamagnetic" experience a force in a magnetic field gradient, but do not become permanently magnetized. Crystals of magnetic iron oxides may be either ferromagnetic or superparamagnetic, depending on the size of the crystals. Superparamagnetic oxides of iron generally

result when the crystal is less than 350 Å(0.035μ) in diameter; larger crystals generally have a ferromagnetic character. Following initial exposure to a magnetic field, ferromagnetic particles tend to aggregate because of magnetic attraction between the permanently magnetized particles, as has been noted by Robinson and others, Biotech. Bioeng., XV: 603–606 (1973).

As described, for example, in U.S. Patent No. 4,604,222, superparamagnetic particles are generally prepared by ball–milling magnetic powders for long periods of time, followed by tedious sieving and purification processes. As a result, they have been extremely costly and this has limited their applications.

For use in biological separation, it is necessary to derivatize the particle so that functional groups, such as amine or carboxyl, are present at the surface for bonding to antibodies and the like. This has required the use of costly reagents, such as amine or carboxyl silanes, and the process of attachment to the magnetic particle is difficult. See U.S. Patent Nos. 4,672,040 and 4,683,032.

The preparation of magnetite by means of hydroxide addition to a solution of ferrous/ferric salts is well known. The concept of using a dispersing agent during or after the preparation to stabilize the magnetite particles has been reported, for example in U.S. Patent No. 4,019,995 and is the basis for a commercial product ("Lignosite") manufactured by the Georgia–Pacific Corporation. However, that product consists of magnetite particles that are appended to the lignin polymer chain and are

not encapsulated. The ratio of lignin to magnetite is quite large, and the product does not appear to be suitable for biological work.

Dispersible magnetic iron oxide particles reportedly having 300 Å diameters and surface amine groups were prepared by base precipitation of ferrous chloride and ferric chloride in the presence of polyethylene imine, according to Rembaum in U.S. Pat. No. 4,267,234. Reportedly, these particles were exposed to a magnetic field three times during preparation and were described as redispersible. The magnetic particles were mixed with a glutaraldehyde suspension polymerization system to form magnetic polyglutaraldehyde microspheres with reported diameters of 0.1μ. Polyglutaraldehyde microspheres have aldehyde groups on the surface which can form bonds to amino-containing molecules such as proteins. However, in general, only compounds which are capable of reacting with aldehyde groups can be directly linked to the surface of polyglutaraldehyde microspheres. Moreover, magnetic polyglutaraldehyde microspheres are not sufficiently stable for certain applications.

Latex particles containing magnetite particles dispersed within the latex sphere are available in the micron (latex) range. See "Uniform Latex Particles" Seragen Diagnostics, Inc., April, 1986, supplement. These are derivatized to be used for antigen separation. Large polyacrylamide-agarose particles containing finely divided magnetite are used for affinity chromatography. These particles are in the several-micron range.

U.S. Patent No. 4,582,622 describes the preparation of a "homogenous" magnetic particulate, having a particle size of 0.8–50 μm by preparing an aqueous colloidal solution containing gelatin, a water soluble polysaccharide such as gum arabic, sodium polymetaphosphate and a ferromagnetic substance, adjusting the pH to 2.5 to 6 with an acid, and forming a water–insoluble particulate by adding an aldehyde. The magnetic particulate is useful as a carrier to immobilize such biological proteins as antigens, antibodies, and enzymes for use in diagnostic assays. As shown in Example 8 of the present application however, the process of that patent, which does not use coacervation conditions as set forth herein, results in coarse aggregates having relatively large particle sizes, compared to the fine, discrete, paramagnetic coacervate coated magnetite particles of the present invention. As will be appreciated by those skilled in the art, smaller particles have larger surface areas per unit weight (or volume) than large particles, which, in turn, affords greater efficiency of use in that less small particle carrier than large particle carrier would be required to carry a given amount of immobilized biological protein. Also, the smaller size particles of the present invention are amenable to ingestion by animals for diagnostic or therapeutic use whereas the larger particles of U.S. Patent No. 4,582,622 would be expected to be unsuitable for such use by virtue of their size. In addition, such large particles are not suitable for magnetic separation techniques such as that described in Example 1 of the present application.

The present invention provides a method for preparing superparamagnetic particles of magnetite (Fe_3O_4) that are encapsulated by a thin layer of modified gelatin. The multifunctionality of the modified gelatin makes the attachment of biologically active compounds such as antibodies very facile. In addition, the encapsulation stabilizes these very small particles.

The present invention also provides superparamagnetic magnetite particles that are encapsulated by a thin shell of a coacervate of gelatin and gum arabic (or another polymeric acid comprising recurring acid groups, preferably those selected from the group consisting of carboxylic acid groups and sulfonic acid groups). This procedure eliminates the need for extensive milling and grinding that have heretofore been widely used to prepare superparamagnetic particles. The encapsulation materials provide reactivity through the various functional groups present in the gelatin–gum arabic shell or coating.

In the drawings:

FIG. 1 is a Sigma H loop showing the magnetization curve for the encapsulated superparamagnetic magnetite particles of Example 1.

In accordance with the present invention, superparamagnetic magnetite particles with a narrow particle size distribution in the range of from 50 Å to 350 Å are prepared by adding a mixture of ferric and ferrous salts to water in amounts that provide a molar ratio of ferric to ferrous ions in the range of 1.6 to 2.4 and in concentrations in the range of 0.01 to 1 molar, preferably 0.05 to 0.5 molar, more preferably 0.1 molar; optionally, adding

acid, for example, sulfuric acid, to adjust the pH to less than 1.5; preferably adding from 0.1 to 5% (wt/vol) of a surfactant solution; preferably purging the resulting solution of oxygen by bubbling therethrough an inert gas, preferably nitrogen, for a period of at least 10 minutes, preferably at least 30 minutes; adding to the (preferably purged) solution (preferably, as rapidly as possible), with stirring, concentrated NaOH (or an equivalent hydroxide, for example, ammonium, potassium, or lithium hydroxide) in an amount in excess of 8 moles of hydroxide ion per mole of Fe^{++} present in the solution, to form particles of Fe_3O_4 having a particle size distribution in the above described range; washing the resultant magnetite particles with water, preferably with the assistance of magnetic separation, until the pH of the magnetite dispersion is within the range of 10-11; depositing a gelatin/polymeric acid coating on the particles by coacervate; and crosslinking. Preferably the coacervation is effected by removing excess water, preferably with the aid of magnetic separation, from the magnetite dispersion that had been washed to pH 10-11 to form a concentrated dispersion of magnetite particles, adding the resulting magnetite dispersion, with stirring, to an aqueous solution, having a temperature of at least 40°C, of gelatin having an isoelectric point greater than 8 and a polymeric acid (preferably gum arabic) comprising at least one recurring acid group, preferably selected from the group consisting of carboxylic acid groups and sulfonic acid groups, each of said gelatin and polymeric acid being present in a concentration of from 1% to 10% (w/vol) and the ratio of magnetite to

the gelatin/polymeric acid mixture on a dry basis b ing from 4:1 to 1:1; and adjusting to coacervation conditions comprising a pH in the range of between 4 and 5.5 and a concentration of the gelatin/polymeric acid mixture of less than 2% (wt/vol); and the crosslinking is effected with a known gelatin hardener.

Preferably, each of the gelatin and gum arabic (or other polymeric acid) is present in the aqueous solution of step (5) in a concentration of 4% (w/vol) and the ratio of magnetite to the gelatin/polymeric acid mixture on a dry basis is preferably 3:1. Preferably, the coacervation is effected by adjusting the pH with H_2SO_4 (or another suitable acid such as acetic acid, or a strong mineral acid, for example, HCl) to a pH of 4.0 to 5.5, more preferably a pH of 4.5; and adding the resulting suspension slowly (over a period of from 5 to 30 minutes) with stirring, to a large excess of cold water maintained at a temperature below 5°C, the resulting suspension being preferably stirred for at least 30 minutes to stabilize the coacervate coated particles. The crosslinking is preferably effected by rapidly adding to the suspension of stabilized coacervate coated particles a concentrated solution of glutaraldehyde or another gelatin hardener in such amount as to provide the equivalent of at least 2 gms. of glutaraldehyde per 100 gm. of gelatin on a dry basis, so as to crosslink the gelatin in the coacervate coating on the magnetic particles; stirring, preferably for 30 minutes, to assure completion of the crosslinking reaction; raising the pH to above 7 with a base such as NaOH; increasing the temperature slowly to 20–25°C (ambient); and washing with water to remove unreacted glutaraldehyde.

Preferably the starting ferric and ferrous salts are sulfates. However, other water soluble salts, such as chlorides or other halides, acetates and nitrates can be used.

While the ratio of ferric to ferrous ion in the process of the present invention may be varied within the range of 1.6 to 2.4, it is presently preferred that the ratio be approximately 2 so as to provide substantially stoichiometric amounts to satisfy the equation:

$$2Fe^{+++} + Fe^{++} + 8(OH)^{-} \longrightarrow Fe_3O_4 + 4H_2O.$$

Although sodium dodecyl sulfate is presently preferred for use as the surfactant in the process of the invention, other anionic surfactants and cationic surfactants are also useful and non-ionic surfactants are expected to be useful. A variety of such surfactants can be selected from McCutcheon's Emulsifiers and Detergents, McCutcheon Division, MC Publishing Co., Glen Rock, New Jersey, USA.

Suitable anionic surfactants include Triton 770, an alkylaryl polyether sulfate, sodium salt, sold by Rohm and Haas Co.; Triton X-200, an alkylaryl polyether sulfonate, sodium salt, sold by Rohm and Haas Co.; Triton GR-5M, dioctyl sodium sulfosuccinate, sold by Rohm and Haas Co.; Sterling AM, an ammonium lauryl sulfonate sold by Canada Packers, Inc.; Gafac RM-710, the free acid of a complex organic phosphate ester sold by GAF Corp.; and Witcolate, an alcohol ether sulfate sold by Witco Chem. Corp.

Suitable cationic surfactants include dodecyltrimethylammonium chloride, Ammonyx DMCD-40, a

lauryldimethyl amine oxide sold by Onyx Chem. Co.; Ammonyx T, a cetyl dimethyl benzyl ammonium chloride also sold by Onyx Chem Co.; Emcol CC55, a polypropoxy quaternary ammonium acetate sold by Witco Chem Corp.; Triton RW-Series, cationic polyalkylene glycols sold by Rohm and Haas Co.; and Emulsifier 3, a quaternary ammonium chloride sold by Tomah Products, Inc.

Suitable non-ionic surfactants include Surfactant 10G, a nonylphenoxypolyglycidol sold by Olin Chem Co.; and various Triton alkylaryloxy polyethoxy ethanols sold by Rohm and Haas Co., such as Triton X-100.

It is preferred to use a substantial excess of hydroxide over the stoichiometric amount in step (2) above, preferably of the order of 10 moles per mole of Fe^{++}. While a ratio of $NaOH/Fe^{++}$ in excess of 10:1 can be used, there does not appear to be an advantage in doing so. While gum arabic is preferably used as the coacervating agent for the gelatin, another polymeric acid comprising recurring acid groups selected from the group consisting of carboxylic acid groups and sulfonic acid groups can be substituted for the gum arabic such as alginic acid, maleic acid, fumaric acid, citraconic acid, itaconic acid, crotonic acid, 3-acrylamidopropane-sulfonic acid, 2-acrylamido-2-methylpropanesulfonic acid, 3-acryloyloxypropanesulfonic acid, styrene-sulfonic acid, and so forth, typical comonomers being alkyl acrylates and alkyl methacrylates such as methyl methacrylate, ethyl acrylate, and vinyl monomers such as ethylene, for example, partially hydrolyzed poly(ethylene-co-maleic anhydride), methyl vinyl ether, styrene, vinyl acetate, for example, partially hydrolyzed poly(vinyl acetate-co-maleic

anhydride), amides such as acrylamide, methacrylamide and N-isopropylacrylamide. The molecular weights of the polymers can range from 5,000 to 300,000.

When gum arabic is used, the preferred weight ratio of gelatin to gum arabic is 1:1, although this ratio can conveniently be within the range of 2:1 to 1:2. When other polymeric acids, preferably polycarboxylic or polysulfonic acids, are substituted for gum arabic, the ratios can be adjusted accordingly.

Similarly, while the presently preferred ratio of magnetite to coacervate on a dry basis is 3:1, this ratio can conveniently be selected within the range of 4:1 to 1:1.

Preferably, the ratio of suspension to cold water in the quenching step described above is 1 liter of suspension to 8-10 liters of cold water.

While glutaraldehyde is the presently preferred crosslinking agent for use in the process of this invention, other gelatin hardeners known to those skilled in the photographic arts can be substituted, with suitable adjustments as may be required to maintain equivalent stoichiometry.

Typical useful gelatin hardeners include formaldehyde and dialdehydes such as succinaldehyde and glutaraldehyde as described in U.S. Patent No. 3,232,764; active esters such as described in U.S. Patent No. 3,542,558; active halogen compounds such as described in U.S. Patents Nos. 3,106,468, 3,305,376 and 3,957,882; s-triazines such as described in U.S. Patent No. 3,325.287; aziridines such as described in U.S. Patent No. 3,575,705; active olefins such as described in U.S. Patents Nos. 3,490,911 and 3,640,720; vinylsulfones such as

bis(vinylsulfonylmethyl) ether and bis(vinylsulfonyl)-methane as described in U.S. Patent No. 3,841,872 and U.S. Patent No. 3,539,644; halogen-substituted aldehyde acids such as mucochloric and mucobromic acids; and polymeric hardeners such as dialdehyde starches poly(acrolein-co-methacrylic acid); poly(styrene-co-2-chloroethylsulfonylmethylstyrene) and poly(styrene-co-vinylsulfonylmethylstyrene).

The coacervate coated superparamagnetic particles of the present invention have a mean diameter in the range of from 70 Å to 450 Å, preferably from 100 Å to 400 Å, more preferably from 150 Å to 350 Å and comprise magnetite particles having a mean diameter in the range of from 50 Å to 350 Å, preferably 100 Å to 300 Å, more preferably 150 Å to 250 Å, that are coated with a coating that is from 20 Å to 100 Å thick, preferably 30 Å to 50 Å thick, which coating comprises a crosslinked coacervate of gelatin with gum arabic or another polymeric acid, preferably one containing repeating units of a carboxylic acid or a sulfonic acid; the magnetite particles, before being coated, having a magnetization of greater than 30 emu/gm, preferably greater than 40 emu/gm, more preferably greater than 50 emu/gm and a coercive force of less than 30 Oe, preferably less than 25 Oe, more preferably less than 20 Oe. The coated particles have a magnetization greater than 30 emu/gm preferably greater than 40 emu/gm and a coercive force less than 30 Oe, preferably less than 25 Oe, more preferably less than 20 Oe. The magnetization and coercive force values of the paramagnetic particles as set forth herein are values obtained by using a VSM meter while applying a magnetic field of 2500 Oe to the dry particles.

As previously indicated, the coated superparamagnetic particles of the invention can be used in known techniques for separation of biological materials, as described, for example, in U.S. Patent No. 4,672,040 and discussed in the Description Relative to the Prior Art hereinabove, as well as in drug delivery systems, for diagnostic imaging and in other applications wherein it is advantageous to use fine superparamagnetic particles having a narrow particle size distribution, particularly where biocompatability is important.

The following examples are presented to illustrate the practice of the present invention:

Example 1

The pH of 100 ml of an equimolar mixture of ferrous and ferric sulfate was adjusted to 1.0 with 25% sulfuric acid. To this was added 10 ml. of a 4% gelatin solution acid processed (pI – 9) whose pH had been adjusted to 0.8 with sulfuric acid solution. The temperature was raised to 50°C and 25% sodium hydroxide solution was added over a period of five minutes to give a final pH of 12.5. During this time, the solution was stirred in the presence of air. The black suspension was separated magnetically and washed with distilled water. The washed magnetite precipitate was then mixed with 100 ml. of a solution containing 4% w/vol of each of gelatin and gum arabic. The gelatin was the same type as used in the magnetite preparation step described above. The mixture of magnetite and gelatin–gum arabic was agitated at 40°C and the pH was lowered to 4.5 with 25% HCl. This mixture was then poured slowly into 500 ml. of water that was agitated rapidly at 4.5°C. After 30 minutes, 20 ml. of 50% glutaraldehyde was

added, and the gelatin coating was considered to be fully crosslinked and the encapsulated magnetite was washed several times by distilled water, using magnetic separation.

Electron microscope examination showed the particles to be 100–150 Å in diameter, with a very minor 40–50 Å fraction. Elemental analysis was used to determine the gelatin–gum arabic content, and estimates of shell thickness of 31 Å were calculated (assuming the magnetite particle diameter to be 100 Å). Magnetic evaluation is shown in Figure 1.

It can be readily seen from the magnetization curve that no hysteresis exists.

Magnetic separation was demonstrated by inserting a plug of steel wool (fine grade) approximately 3 cm long and 1.5 cm ID into the stem of a small plastic powder funnel. The stem was placed between the poles of a small horseshoe magnet (800 gauss), and the encapsulated magnetite solution poured into the funnel. Clear liquid drained out. Removing the magnet and pouring the clear liquid into the funnel caused the particles to be removed from the steel wool. The recovery was excellent, which is further proof that the particles are superparamagnetic.

Example 2

Example 1 was repeated, but 0.5% of dodecyltrimethylammonium chloride was used as a surfactant/dispersing agent instead of gelatin in the preparation of the magnetite. The encapsulation step involving gelatin and gum arabic was carried out exactly as described in Example 1, and the results were comparable.

Example 3

This example illustrates the hydrophobizing of the gelatin-gum arabic shell so that the encapsulated Fe_3O_4 particles have an affinity for non-water-miscible organic solvents such as toluene or ethylbenzene, thus making them useful in non-aqueous systems such as ferro fluids. (See, for example, U.S. Patent No. 3,531,413.)

One gram of the wet coagulum of Example 1 was mixed with 15 ml. of water and 1 ml. of Quilon M (DuPont), and the mixture was shaken for five days. Another sample, which consisted of the unencapsulated magnetite preparation was also treated in this manner. The samples were then decanted and rinsed several times with water, decanting magnetically between rinses. They were then rinsed three times with methanol, decanting magnetically between rinses, after which they were mixed with ethylbenzene. Comparison with untreated samples of encapsulated magnetite and unencapsulated magnetite particles that were washed in the same manner showed that only the Quilon treated encapsulated magnetite had much slower sedimentation rates. This indicates that the Quilon had reacted with the surface of these magnetic samples and had attached so that the surfaces were now hydrophobic.

Quilon is a chrome complex sold by the DuPont Corporation in which myristic acid is coordinated with trivalent chromium. The commercial solution (in isopropanol) contains 5.7% (by weight) chromium and 11.7% fatty acid. The anion is chloride (7.8%). Quilon is generally used to impart water repellency to paper and fabric.

Other hydrophobizing agents such as alkyl titanites, silanes and borates could also be used.

Example 4

A suspension of magnetite particles that had been encapsulated by gelatin–gum arabic coacervate as described in Example 1 was prepared mixing 2.34 grams of a concentrated dispersion (13% wt/vol, dry basis) of the particles with 25 ml. of distilled water. This was stirred for 3 minutes in a high speed (Virtis) mixer at 23,000 rpm for 3 minutes. Twenty–five ml. of a 2% solution of benzoquinone was added and the pH raised to 11.1 with sodium hydroxide. This mixture was shaken for 18 hours, after which it was magnetically separated and washed three times with distilled water, followed by two washes with methanol. It was dried at 75°C. Combustion analysis showed that the gelatin–gum arabic shell had reacted with the benzoquinone so that 14% of the modified shell was benzoquinone.

Example 5

A suspension of encapsulated magnetite was prepared as described in Example 1 and mixed with a solution of glutaraldehyde (1%). This was shaken for 2 1/2 days after which it was magnetically separated and washed with water (3 cycles). The washed particles were mixed with a 2% gelatin (isoelectric point 8.3) solution and stirred at 40°C for 18 hours. It was then magnetically separated and washed 3 times with 35° distilled water. This was followed by two methanol washes. Analysis showed that the gelatin–gum arabic shell had doubled in weight due to the coupling of gelatin in the solution with the glutaraldehyde activated particle surface.

Example 6

A suspension of encapsulated magnetite was prepared as described in Example 1 and mixed with a

solution containing 2% gum arabic and 5% butanediol bis(glycidyl) ether. This was shaken for 6 days after which it was separated magnetically and washed with water. This was followed by a methanol wash. Analysis showed that the particles had reacted with a substantial amount of gum arabic through the epoxide coupling to the encapsulated magnetite particle. The shell had undergone a 50% increase in weight.

Example 7

Two hundred ml. of a solution that is 1 molar in both ferrous and ferric sulfate were prepared and the resultant mixture made 1% in sodium dodecyl sulfate. The solution was purged of dissolved oxygen by passing nitrogen through for 30 minutes at 25°C. To the rapidly stirred mixture, 40 gms. of sodium hydroxide in 80 ml. water was rapidly added and the stirring continued under nitrogen for one hour. After this period of time, the reaction mixture was poured into an excess of water. The magnetite was separated magnetically and washed with distilled water until the pH of the wash water was below 11. The reaction mixture was identified as Fe_3O_4 by means of X-Ray diffraction. The magnetization and coercive force values were: 56.9 emu/g and 15.4 Oe respectively. A coercive force below 30 Oe is indicative of a superparamagnetic material.

The wet magnetite paste was dispersed in 200 ml. of a gelatin-gum arabic solution in which each of the gelatin and gum arabic was present in a concentration of 4% wt/vol. The gelatin had an isoelectric point of approximately 8.5. The mixture was agitated at 40°C and the pH lowered to 4.5 with 25% sulfuric acid. This mixture was then poured

slowly into 2 liters of water that was agitated rapidly at 4°C. After 30 minutes, 4 ml. of 50% glutaraldehyde was added and the suspension of coacervated magnetite was stirred for an additional 30 minutes at 4°C. The pH was then raised to 10 with sodium hydroxide and the solution allowed to come to room temperature. At this point, the coacervate shells were fully crosslinked, and the encapsulated magnetite was washed several times with distilled water at room temperature using magnetic separation between the washes. Electron microscopic examination showed the particles to be 100–150 Å in diameter with a very minor 40–50 Å fraction. Elemental analysis was used to determine the gelatin–gum arabic content and estimates of shell thickness of 31 Å were calculated assuming the magnetite particle diameter to be 100 Å.

Example 8

U.S. Patent No. 4,582,622 describes the encapsulation of magnetic particles by a process which involves the deposition of a mixture consisting of gelatin, gum arabic, and sodium polymetaphosphate. The procedure set forth in Example 1 of the patent was followed with the substitution of sodium dodecyl sulfate for the surfactants there used (alkylsulfomaleate, sodium oleate, and Demol Ep).

Three variations with regard to the quantity of magnetite were made: (1) the concentration used in Example 1 of the patent, which is very low, (2) a 5 fold and (3) a 10 fold increase therefrom.

All particles prepared showed very coarse aggregation with dimensions ranging from 1 to 10 microns. Separation using the steel wool funnel as described in Example 1 (of this application) was

unsuccessful in that the particles could not be washed off the steel wool after the magnetic field had been removed.

CLAIMS:

1. A method for preparing stable, coated superparamagnetic magnetite particles having a narrow particle size distribution which comprises forming an aqueous solution of ferric and ferrous salts by adding a mixture of ferric and ferrous salts to water in amounts that provide a molar ratio of ferric to ferrous ion in the range of from 1.6 to 2.4 and in concentrations in the range of from 0.1 to 1 molar; adding to the solution concentrated hydroxide in an amount in excess of 8 moles of OH^- per mole of ferrous ion present in the solution to form a dispersion of fine particles of magnetite; washing the resultant magnetite particles with water until the pH of the magnetite dispersion is within the range of 10–11; depositing a coating of gelatin and a polymeric acid on the particles by coacervation; and then crosslinking.

2. The method of Claim 1 which further comprises adding sufficient acid to the aqueous mixture of ferric and ferrous salts to adjust the pH to less than 1.5.

3. The method of Claim 1 which further comprises adding from 0.1 to 5% (wt/vol) of a surfactant to the salt solution.

4. The method of Claim 1 which further comprises purging the solution of oxygen by bubbling therethrough an inert gas for a period of at least 10 minutes.

5. The method of Claim 1 wherein the gelatin/polymeric acid coating is deposited on the magnetite particles by removing excess water from the magnetite dispersion that had been washed to said pH of 10–11, to form a concentrated dispersion of

magnetite particles, then coating the thus produced particles by adding the resulting magnetite dispersion to an aqueous solution, having a temperature of at least 40°C, of gelatin having an isoelectric point greater than 8 and a polymeric acid comprising at least one recurring acid group.

6. The method of Claim 5 wherein the gelatin/polymeric acid mixture is coacervated by adjusting to coacervation conditions comprising a pH in the range of between 4.0 and 5.5 and a concentration of the gelatin/polymeric acid mixture of less than 2% (wt/vol).

7. The method of Claim 5 wherein the coacervate is crosslinked with a suitable crosslinking agent for gelatin.

8. Encapsulated superparamagnetic particles having a narrow particle size distribution, the mean diameter of the particles being within the range of from 70 Å to 450 Å, said particles comprising particles of magnetite having a narrow particle size distribution, the mean diameter of the magnetic particles being between 50 Å and 350 Å, said magnetite particles having a magnetization of greater than 30 emu/gm and a coercive force of less than 30 Oe, said magnetite particles being encapsulated with a coating of a crosslinked coacervate of gelatin and a polymeric acid.

9. The superparamagnetic particles of Claim 8 wherein said polymeric acid comprises recurring acid groups selected from the group consisting of carboxylic acid groups and sulfonic acid groups.

10. The encapsulated particles of Claim 8 wherein the magnetite particles have a magnetization greater than 50 emu/gm and a coercive force less than 25 Oe.

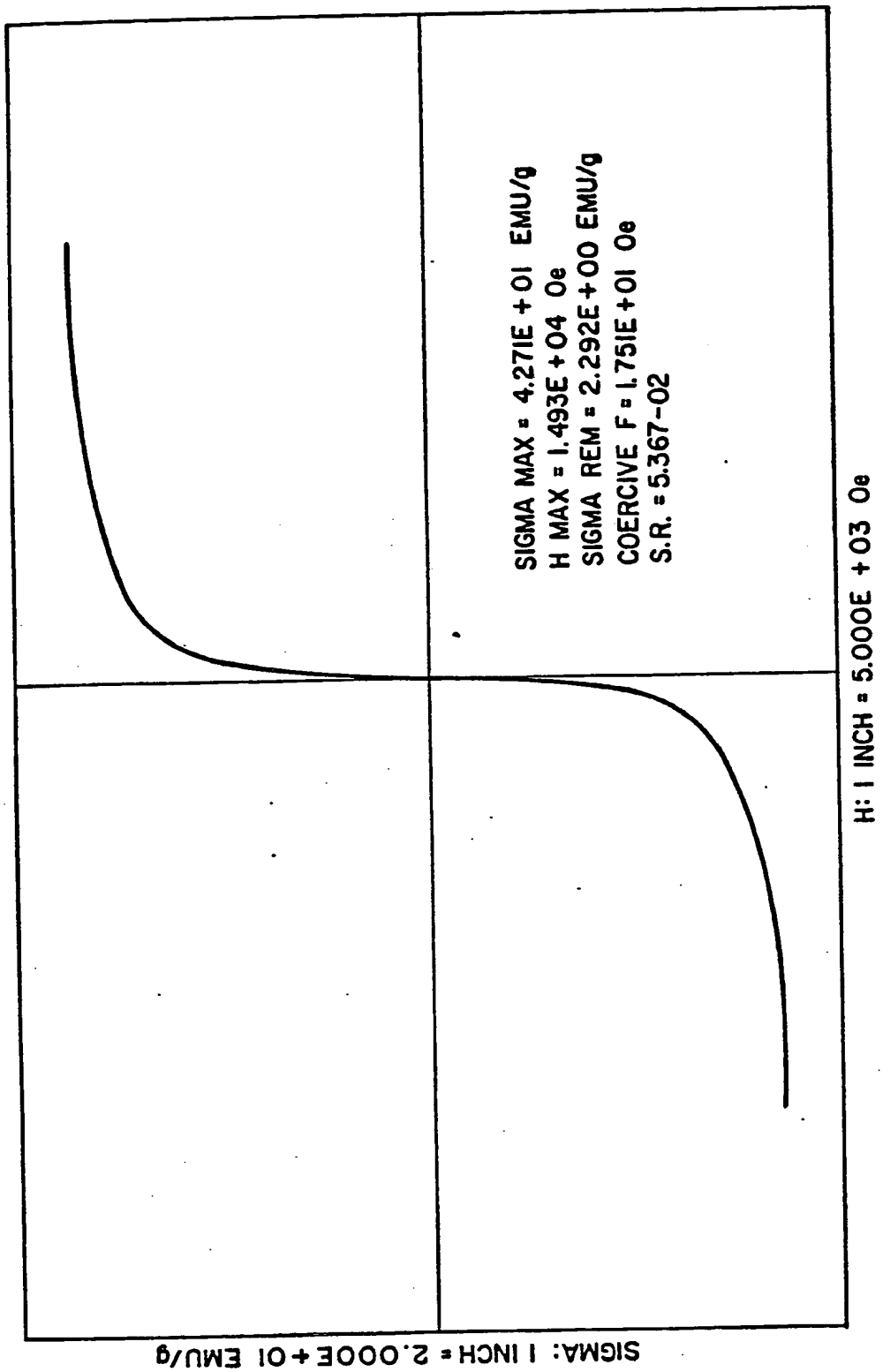
SIGMA MAX = 4.271E+01 EMU/g
H MAX = 1.493E+04 Oe
SIGMA REM = 2.292E+00 EMU/g
COERCIVE F = 1.751E+01 Oe
S.R. = 5.367-02
H: 1 INCH = 5.000E+03 Oe
SIGMA: 1 INCH = 2.000E+01 EMU/g

FIG. 1

INTERNATIONAL SEARCH REPORT

International Application No PCT/US 89/01901

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [6]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC[4]: H 01 F 1/37,// B 03 C 1/00, G 01 N 33/543

II. FIELDS SEARCHED

Minimum Documentation Searched [7]

Classification System	Classification Symbols
IPC[4]	H 01 F, B 03 C

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

Category *	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No. [13]
Y	IEEE Transactions on Magnetic, vol. 24, no. 2, March 1988, IEEE, (New York, US), A. Wooding et al.: "Proteins and carbohydrates as alternative surfactants for the preparation of stable magnetic fluids", pages 1650-1652 see page 1650	1,9
A	--	3,4,8
Y	US, A, 4169804 (A.F. YAPEL) 2 October 1979 see claims 1,2,3,7,9,10,12,14,15; column 4, line 53 - column 5, line 36; column 6, lines 52-53	1,9
A	--	7
A	US, A, 4452773 (R.S. MOLDAY) 5 June 1984 see claims 1,2,13,15,19,20	1,8
	-- ./.	

* Special categories of cited documents: [10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
4th September 1989	02 OCT. 1989
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT OFFICE	T.K. WILLIS

International Application No. PCT/US 89/01901

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category *	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No
A	EP, A, 0184710 (BAYER AG) 18 June 1986 see claims 1,10 --	1,7
A	FR, A, 2334106 (INSTITUT PASTEUR) 1 July 1977 --------	

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO. US 8901901 SA 29110

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report. The members are as contained in the European Patent Office EDP file on 26/09/89
The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

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US-A- 4169804	02-10-79	None	
US-A- 4452773	05-06-84	CA-A- 1217934	17-02-87
EP-A- 0184710	18-06-86	DE-A- 3444939	12-06-86
		JP-A- 61140523	27-06-86
FR-A- 2334106	01-07-77	DE-A,C 2654723	23-06-77
		GB-A- 1577956	29-10-80
		JP-A- 52082723	11-07-77
		SE-A- 7613487	03-06-77
		SE-B- 441393	30-09-85
		SE-A- 8000427	17-01-80